

EXPERIMENTS ON THE ORIGIN OF MOLECULAR CHIRALITY BY  
PARITY NON-CONSERVATION DURING  $\beta$ -DECAY

William A. Bonner  
 Department of Chemistry, Stanford University  
 and  
 Ames Research Center, NASA

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SUMMARY

Experiments are described to test a theory for the origin of optical activity wherein the longitudinally polarized electrons resulting from parity violation during radioactive  $\beta$ -decay, and their resulting circularly polarized Bremsstrahlung, might interact asymmetrically with organic matter to yield optically active products. The historical background to this subject is briefly reviewed. Our experiments involve subjecting a number of racemic and optically active amino acid samples to irradiation in a 61700 Ci  $^{90}\text{Sr}$ - $^{90}\text{Y}$   $\beta$ -radiation source for a period of 1.34 years (total dose:  $4.11 \times 10^8$  rads), then examining them for any asymmetric effects by means of optical rotatory dispersion and analytical gas chromatography. In the cases of D, L-leucine, norleucine, norvaline and proline as solids, of D, L-leucine in solution (neutral and as Na and HCl salts) and of D, L-tyrosine in alkaline solution no optical rotation was observed during ORD measurements in the 250-630 nm spectral region. While slight differences were noted in the percent radiolysis of solid D- (12.7%) and L-leucine (16.2%) as determined by GC, no enrichment of either enantiomer was found by GC analyses of irradiated D, L-leucine, either as a

Postal address of author: Dr. William A. Bonner, Department of Chemistry,  
 Stanford University, Stanford, CA 94305 U.S.A.

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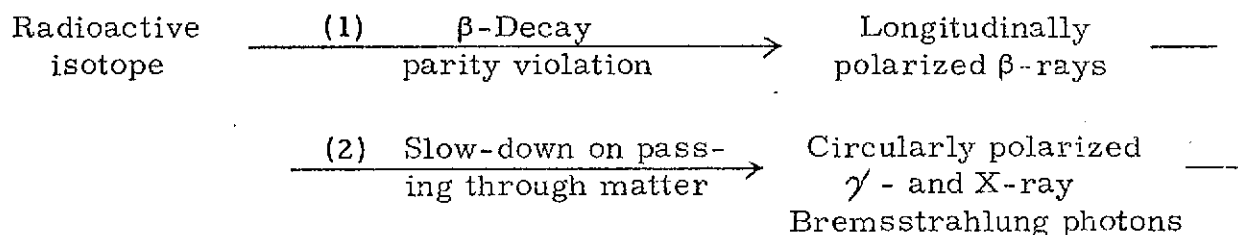
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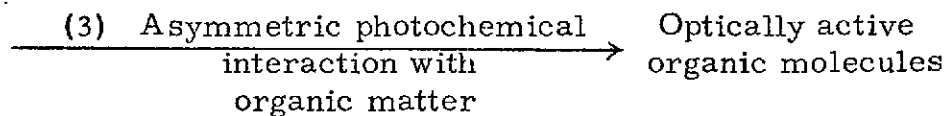
solid (13.7% radiolyzed) or as its Na salt (48.6% radiolyzed) or HCl salt (34.6% radiolyzed) in aqueous solution. Several explanations are offered for our experimental findings.

## INTRODUCTION

The question of the origin of optically active molecules in nature, a question intimately connected with that of the origin of life itself, is one which has fascinated scientists since the time of Pasteur. Since that time a number of theories have been proposed -- sometimes with attempted experimental verification -- to explain the natural origin of optically active molecules abiotically (Bonner, 1972a), and in recent years optical activity has been suggested as a perhaps unique criterion for the recognition of life elsewhere in the universe (Halpern et al., 1966; Stryer, 1966; Fox, 1966). We have been interested in the experimental investigation of various mechanisms for the abiotic generation of optically active molecules for two reasons: 1) some of the "positive results" reported in earlier literature are so close to experimental error as to be ambiguous and might be made more definitive with modern techniques and instrumentation; and 2) if net molecular chirality proved to be the inevitable consequence of some universal physical process, then optical activity per se would be an inadequate criterion for extraterrestrial life. The question is thus of importance to this aspect of future space exploration.

A novel mechanism which should in principle lead inevitably to products having a unique molecular chirality was first suggested by Vester (1957), and involved a consequence of the then recently predicted (Lee and Yang, 1956) violation of the parity principle during  $\beta$ -decay of certain radioactive isotopes. Ulbricht (1959, 1962) subsequently summarized this proposed mechanism in roughly the following format:





It should be noted that, in principle at least, each step in this proposed mechanism has previously had independent experimental confirmation. Thus Wu and coworkers (1957) confirmed the prediction of Lee and Yang (1956) that the electrons emitted during  $\beta$ -decay (step (1)) were longitudinally polarized. Goldhaber et al. (1957) investigated the degree and sense of circular polarization of the external Bremsstrahlung from  $^{90}\text{Sr}$   $\beta$ -rays (step (2)) and found that at the high energy end of the Bremsstrahlung spectrum the photons emitted were almost completely circularly polarized. Finally, the asymmetric interaction of circularly polarized photons with organic matter (step (3)), originally demonstrated by the classic studies of Kuhn and coworkers (1929, 1930a, 1930b) in the ultraviolet region, has recently been confirmed (again with ultraviolet wavelengths only) by Bernstein et al. (1973) and by Kagan and coworkers (1971). Ulbricht and Vester (1962) calculated, however, that even under favorable circumstances the optical activity generated via the above mechanism might not be measurable within reasonable time spans and that a "source of 1 Curie, complete absorption of the  $\beta$ -radiation by the reactants, a product with a specific rotation of  $100^\circ$ , and a quantum yield of 1, might produce measurable optical activity in a period on the order of one year." Despite these anticipated difficulties Vester, Ulbricht and Krauch (1959) and Ulbricht and Vester (1962) undertook an experimental investigation of the above mechanism, examining a large number of synthetic and degradative organic reactions which would yield optically active organic products if the above effects were valid and of sufficient magnitude. Some ten different reactions were conducted in the presence of a variety of  $\beta$ -emitters, (e.g.,  $^{32}\text{P}$ ,  $^{90}\text{Sr}$ - $^{90}\text{Y}$ ,  $^{152}\text{Eu}$ ,  $^{108}\text{Ag}$ - $^{110}\text{Ag}$  of various radioactivity levels (30-2500 mCi) for differing time intervals (5-2900 min.) and at varying temperatures ( $-50^\circ$  to  $50^\circ\text{C}$ ). Optical activities of the products of some 36 separate experiments were measured on two different precision polarimeters, averaging a minimum of 10 readings for each measurement. The observed rotations in each of the experiments proved to be within experimental error of zero, however, and the authors concluded that any optical

activity produced was less than 0.02% and that definitive results would at least require stronger  $\beta$ -sources and longer irradiation times.

More recently Gol'danskii and Khrapov (1963) have conducted a detailed investigation of the effects of electron irradiation on both the optical inactivity of solid racemates and the optical activity of optical antipodes, employing some 22 different optically active or racemic organic substrates. The electron sources used were the  $\beta$ -decay electrons from  $^{104}\text{Rh}$  as well as electrons from both a microtron and a cascade accelerator. Using a Hilger precision polarimeter with sodium D-line light as their analytical tool, these investigators found that in none of their experiments was optical activity induced in their irradiated racemic samples, nor could differences in the action of the electrons on their optically active antipodes be observed.

In 1968 A. S. Garay reported what appeared to be the first positive finding in this area of experimentation. He irradiated D, L-alanine, D, L-tryptophane and D, L-tyrosine with a 0.5 mCi  $^{32}\text{P}$  source under sterile conditions. After a month no measurable optical activity was noted and the experiment was modified. D- and L-Tyrosine in aqueous ethanol containing alkali were irradiated separately with about 0.36 mCi of  $^{90}\text{SrCl}_2$  dissolved in each mixture as the  $\beta$ -ray source, whereupon the ultraviolet absorption spectrum of each solution was measured on a periodic basis over some 1.5 years. Each enantiomer displayed a similar spectrum up to 12 months, but after 18 months the absorption bands of the D-tyrosine were considerable more eradicated than those of the L-tyrosine, suggesting that the former had undergone more degradation than the latter. These differences were not observed in acidic solution, or in control experiments using non-radioactive  $^{88}\text{SrCl}_2$  in alkaline solution. Garay therefore suggested that the decomposition of tyrosine in alkaline solution was actually an oxidative degradation which was being asymmetrically enhanced by the  $\beta$ -rays used or by their Bremsstrahlung.

Garay's report thus constitutes the first and only positive result bearing on the generation of optical activity by way of the Vester-Ulbricht  $\beta$ -decay mechanism. Despite these positive findings and negative control results we have felt some slight misgivings about Prof. Garay's experiment. In the first

place the  $\beta$ -source was relatively weak, only about 0.36 mCi of  $^{90}\text{SrCl}_2$ . Secondly the intended reaction was not primarily induced by the  $\beta$ -rays or their Bremsstrahlung, but only presumed to be in some way enhanced by them. Thus the effect was noted only in alkaline, but not acidic solution. Thirdly, the reaction was conducted in aqueous-ethanol solvent, where solvent photochemistry or radiochemistry (which would involve symmetrical radical intermediates) would be superimposed upon and might obscure any actual asymmetric  $\beta$ -ray or Bremsstrahlung effects. Lastly, we felt that mere differences in the shapes of two ultraviolet absorption spectra were hardly the best or most convincing criterion for the generation of optical activity. For these reasons we decided to repeat Garay's type of experiments, with considerable modifications which would hopefully circumvent some of the above drawbacks. This paper describes the design and rationale of these experiments, and an interim report on their results.

### EXPERIMENTAL

$\beta$ -Ray Source.  $^{90}\text{Sr}$ - $^{90}\text{Y}$  is a pure  $\beta$ -emitter which is available commercially packaged at various radioactivity levels. Our original plan was to irradiate individual racemic amino acids as solid samples by placing them between two 5 Ci wafers of  $^{90}\text{Sr}$ - $^{90}\text{Y}$ , then -- after a period of time -- to examine the partially degraded samples for optical activity. In estimating the lead shielding which would be required for such a source, the Health Physics Department at Stanford University had occasion to calculate the Bremsstrahlung dose rate which this source would provide. This proved to be so low, however, that it became clear (based on the work of Tolbert et al. (1962) and Bonner (1972b)) that inordinate time intervals (tens of years) would be required before significant degradation could be anticipated by the Bremsstrahlung produced. In searching for a more powerful  $\beta$ -ray source, we subsequently contacted the Isotopes Division of Oak Ridge National Laboratory and learned that they had 61,700 Ci of  $^{90}\text{Sr}$ - $^{90}\text{Y}$  oxide retained in dead storage in four approximately 6.4 x 23 cm stainless steel cans immersed under 3 m of water. Since this material was not to be processed for

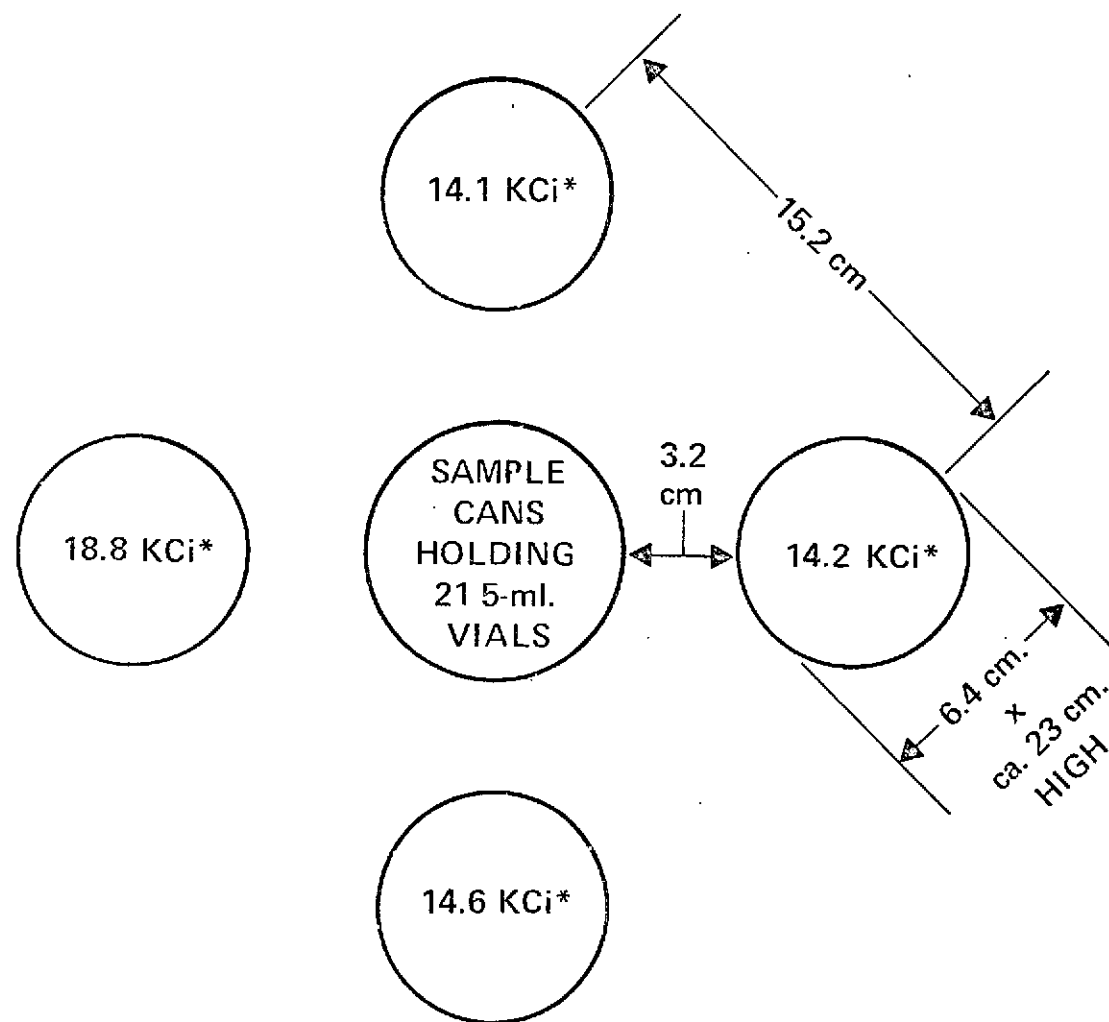
commercial use until an indefinite date in the future, the Isotopes Division contracted to arrange these cans in a concentric pattern, to calibrate the Bremsstrahlung dose rate at the center of this array ( $3.56 \times 10^4$  rads/hr as of Feb. 17, 1971), and to irradiate samples provided by us by placing them at the center of the array for desired time intervals. Fig. 1 shows the details of this 61.7 KCi  $^{90}\text{Sr}$ - $^{90}\text{Y}$  source, which was subsequently used in the experiments described below.

(Insert Fig. 1)

Sample Holder and Samples Irradiated. Our original intention was to irradiate solid amino acid samples individually for specific time intervals. With the space available at the center of the 61.7 KCi source shown in Fig. 1, however, it was clear that a much larger number of samples could be irradiated under a variety of physical conditions, and also that replicate samples could be irradiated for several successive time intervals. Accordingly, a simple sample holder was devised, consisting of an open lead-weighted stainless steel "cage" (two round platforms attached by four long, fully threaded bolts) capable of holding three 5.1 x 8.3 cm (diameter) machined aluminum cans having wall thicknesses of 1 mm. Each can was designed to hold upright twenty-one 5 ml glass sample vials, each equipped with an aluminum screw top having asbestos and lead foil gaskets. After the vials were installed, each can was closed by a tight fitting machined aluminum bottom secured and made watertight by sealing with epoxy resin. The three cans were stacked and clamped in the holder cage, and the array was lowered into the central sample area of the 61.7 KCi  $^{90}\text{Sr}$ - $^{90}\text{Y}$  source on March 18, 1971. At later dates each can could be retrieved from the source individually, thus providing three identical sets of 21 experiments which could be irradiated for three increasing time intervals. The experiments in each of the above three irradiation cans consisted of vials containing the samples listed in Table 1.

(Insert Table I)

The rationale for certain of the experiments listed in Table I was as follows. To aid us in choosing the amino acids to be irradiated, we had access



\*AS OF JAN., 1970.

Fig.1. Detail of 61.7 KCi  $^{90}\text{Sr}$ - $^{90}\text{Y}$   $\beta$ -Radiation Source.

Table I  
SAMPLES PLACED IN  $^{90}\text{Sr}$ -IRRADIATION CANS

No.	Contents of Vial
1	D, L-leucine, solid (0.6 g)
2	D-leucine, solid, (0.5 g)
3	L-leucine, solid, (0.5 g)
4	D, L-norleucine, solid, (0.5 g)
5	D, L-norvaline, solid, (0.5 g)
6	D, L-proline, solid, (0.5 g)
7	D-leucine (0.06 g) in 1:9 ethanol:water (4 ml)
8	L-leucine (0.06 g) in 1:9 ethanol:water (4 ml)
9	D, L-leucine (0.02 g) in 1:9 ethanol:water (4 ml)
10	D, L-leucine (0.35g) in 1N NaOH (2.8 ml; 5% excess)
11	D, L-leucine (0.35 g) in 1N HCl (2.8 ml; 5% excess)
12	D-leucine (0.35 g) in 1N NaOH (2.8 ml; 5% excess)
13	L-leucine (0.35 g) in 1N NaOH (2.8 ml; 5% excess)
14	D-tyrosine (0.002 g) in 80% ethanol (2.5 ml), 2N NaOH (0.2 ml)
15	L-tyrosine (0.002 g) in 80% ethanol (2.5 ml), 2N NaOH (0.2 ml)
16	D, L-tyrosine (.02 g) in 1N NaOH (2 ml)
17	10% Aqueous ammonium acetate solution (3 ml)
18	Glycine (0.2 g) in 37% aqueous formaldehyde (4 ml)
19	37% Aqueous formaldehyde (4 ml), 0.1 N NaOH (0.1 ml)
20	Glycine ethyl ester hydrochloride (0.4 g), isobutylene (0.14g), methanol (3 ml), acetone (1 ml)
21	L-leucine (0.06 g), imidazole (0.03 g) in 1:9 ethanol:water (4 ml)



to a manuscript of Tolbert and coworkers (1962) who studied the radiolysis of solid amino acid samples in a 1000 Ci  $^{137}\text{Cs}$   $\gamma$ -ray source at the University of Colorado. This investigation indicated that leucine, norleucine, norvaline and proline were among the amino acids which showed the highest G(-M) values (number of molecules decomposed per 100 eV of absorbed radiation), that is, they were the most subject to radiolytic destruction. We accordingly selected optically active and racemic samples of these amino acids to study both in the solid state and in solution (Nos. 1-13, Table I). Experiments 14 and 15 in Table I were planned to simulate Garay's (1968) experiments with D- and L-tyrosine, and No. 16 was intended to extend these observations to D, L-tyrosine under different solvent and concentration conditions. Experiment 17 was included as an attempt to amplify the experiments of Paschke *et al.* (1957) on the synthesis of amino acids by the  $\gamma$ -irradiation of ammonium carbonate. No. 18 was included to study a possible radiochemical modification of Akabori and coworkers' (1956) conversion of glycine into serine using formaldehyde. No. 19 involved the well-known "formose" reaction converting aqueous formaldehyde into sugars, and No. 20 represented an attempt to extend Elad and Sinnreich's (1965) photochemical alkylation of glycine derivatives using asymmetric  $\beta$ -ray Bremsstrahlung instead of ultraviolet light. In the present report we focus our attention on experiments No. 1-6, 9, 10, 11 and 16.

Criteria for Optical Activity. For the racemic amino acid samples irradiated (Table I), either as solids or in solution, we have used two criteria to recognize any optical activity developed by the asymmetric degradation of the two enantiomers: 1) measurement of an optical rotatory dispersion curve for each crude, partially degraded sample remaining after irradiation, and 2) conversion of such crude samples to suitable derivative mixtures, followed by assay for the two enantiomeric (or diastereomeric) components in these mixtures by quantitative gas chromatography.

Optical Rotatory Dispersion. ORD curves for solid D, L-samples No. 1, 4, 5 and 6 in Table I were measured at ca. 6.6% concentration in 5 N HCl solution within the wavelength region 250-630 nm, using 2 mm to 5 cm

cells in a Jasco ORD/UV-5 recording spectropolarimeter. In each case the ORD curve for the corresponding authentic unirradiated D, L-sample was measured consecutively under the same conditions for comparison purposes, and all ORD curves were compared with a 5N HCl solvent blank curve measured simultaneously. In Nos. 9, 10, 11 and 16 the recovered sample solutions were subjected directly to similar ORD measurements, using the corresponding authentic D, L-amino acids at the same concentrations in the same solvents (ethanol:water, 1N NaOH or 1N HCl) to provide comparison ORD curves. All unirradiated D, L-comparison samples were obtained from the same original bottles as the irradiated samples and all solutions (except solvent blanks) were filtered through 0.45  $\mu$ m Millipore filters (Millipore Corp., Bedford, Mass.) prior to ORD measurements.

Gas Chromatography. The enantiomeric composition of the irradiated D, L-samples No. 1, 10 and 11 in Table I were determined gas chromatographically by one or more of four methods: 1) Method GC-1: Conversion of the irradiated D, L-Leu or Leu salt sample into its N-trifluoroacetyl (+)-2-butyl ester derivative (Pollock et al., 1965), followed by quantitative analysis of the diastereomeric mixture on a 150' x 0.02" capillary column loaded with carbowax 20M phase (Applied Science Laboratories, Inc., State College, Penn.); conditions: 122° isotherman, He flow rate ca. 3 ml/min. 2) Method GC-2: Similar conversion to N-TFA (+)-2-butyl ester derivatives, followed by similar analysis using a 150' x 0.02" capillary column loaded with Ucon 75 H90,000 phase (Perkin Elmer Corp., Norwalk, Conn.); conditions: 110° isothermal, He flow ca. 3 ml/min. 3) Method GC-3: conversion of the irradiated Leu sample into its N-TFA isopropyl ester, followed by quantitative analysis of the enantiomer mixture on a 7' x 1/8" packed column containing 60-80 mesh Chromosorb W impregnated with 7% N-lauroyl-L-valyl-t-butylamide phase (Miles Laboratories, Inc., Kankakee, Ill.) (Feibush, 1971; Gil-Av, 1973); conditions: 100° isothermal, He flow ca. 10 ml/min. 4) Method GC-4: Similar analysis of the N-TFA isopropyl ester derivative on a 7' x 1/16" packed column containing the same 7% N-lauroyl-L-valyl-t-butylamide phase on Chromosorb W; conditions: 120° isothermal, He flow ca. 3 ml/min. Under

all of these conditions the diastereomers or enantiomers were separable with baseline resolution. In methods GC-1 and GC-2 the diastereomer peak maxima were separated by ca. 1.2 and 2.8 min., respectively; in GC-3 and GC-4 the enantiomer peak maxima separations were ca. 6.9 and 4.7 min., respectively. Average elution times for the two peaks in the four methods were approximately 20, 39, 23 and 18 min., respectively. The analyses were conducted using a Hewlett Packard 5700A gas chromatograph, and peak area integration was accomplished with an Autolab 6300 digital electronic integrator while monitoring with the aid of a Varian Aerograph A25 20-speed recorder. All analyses were run repetitively 3 to 7 times, along with a comparable number of control analyses using derivatives prepared from unirradiated D, L-leucine from the same original bottle. Irradiated sample and control analyses were run in "back-to-back" alternation on a single day to minimize potential discrepancies due to instrumental artifacts. The precision of such gas chromatographic analyses in the case of leucine enantiomer mixtures converted to their N-TFA L-prolyl dipeptide diastereomers has previously been investigated by Bonner (1972b). Similar precision (i. e., ca. 0.1-0.5%) has been found to apply also to the presently described G. C. analyses.

Criteria for Extent of Degradation. The extent of radiolysis suffered by the irradiated leucine samples No. 1, 2, 3, 10 and 11 (Table I) were measured by the gas chromatographic "enantiomeric marker" technique (Bonner, 1973a). This involved adding a known weight of one of the leucine enantiomers as an internal standard to a known weight of the irradiated sample, converting the mixture to one of the above volatile derivatives, and analyzing as before for the fraction of each enantiomer present. The latter data then permit calculation of the amount of undecomposed sample remaining after irradiation, and hence the extent of radiolysis. For samples No. 1, 2, 10 and 11 L-Leu was used as the enantiomeric marker, while D-Leu was employed in No. 3. In samples No. 10 and 11 an aliquot of the irradiated solution was lyophilized to dryness and weighed prior to adding the L-Leu marker. In earlier pilot studies this technique was developed to estimate the extent of radiolysis of D, L- and L-Leu caused by  $\gamma$ -ray irradiation for varying time intervals in a 1270 Ci  $^{60}\text{Co}$  source at Ames Research Center (Bonner, 1973a).

## RESULTS

The first can of samples was recovered from the  $^{90}\text{Sr}$  source after 2976 hours (total dose:  $1.05 \times 10^8$  rads). Examination of the solid D- and L-leucine samples for percent degradation (Table III) indicated the D-Leu was 3.5% and the L-Leu 4.9% decomposed (G(-M) values of 2.5 and 3.4, respectively). Measurement of the optical rotation of the D, L-leucine (No. 1, Table I) (Perkin Elmer Model 421 photoelectric polarimeter) (average of 24 readings for sample solution and for zero point) indicated  $[\alpha]_{\text{D}}^{25} +0.079 \pm 0.022^\circ$  (c, 6.27; 5N HCl), while the D, L-Leu standard showed  $[\alpha]_{\text{D}}^{25} +0.121 \pm 0.021^\circ$  under identical conditions. This constitutes a net  $[\alpha]_{\text{D}}^{25} +0.042 \pm 0.030^\circ$  for the irradiated sample. Assuming the above 3.5% decomposition for D- and 4.9% for L-leucine, one can calculate that the irradiated D, L-Leu should have a D/L ratio of 50.4/49.6. Since  $[\alpha]_{\text{D}}^{25}$  of pure L-Leu is  $+16.0^\circ$  (5 N HCl) (Meister, 1965), the irradiated sample No. 1 should thus show  $[\alpha]_{\text{D}}^{25} -0.118^\circ$ . One thus concludes that sample No. 1 is in fact optically inactive within experimental error. Similarly, ORD measurements on sample No. 1 and a D, L-Leu standard failed to show any departures beyond experimental error from  $\alpha = 0.00^\circ$ , down to 250 nm. Clearly longer irradiation times would be required for unambiguous observable results.

The second can of samples was accordingly retrieved after a total of 11736 hours irradiation (1.34 years; total dose:  $4.11 \times 10^8$  rads). ORD measurements were conducted on the racemic solid samples Nos. 1, 4, 5 and 6 in Table I and on the dissolved racemic samples Nos. 9, 10, 11 and 16. In no case was an ORD curve obtained which was not identical within experimental error to that measured within a similar wavelength span for the corresponding non-irradiated control sample. Since the ORD measurements thus showed all of the irradiated racemic amino acid samples still to be void of optical activity, attention was turned to the application of gas chromatographic criteria to the solid and dissolved Leu samples Nos. 1, 2, 3, 10 and 11 in Table I.

Table II shows the gas chromatographically determined percent degradation and enantiomeric composition of the Na and HCl salts of D, L-Leu

after irradiation in aqueous solution. We note that even after total degradations to the extent of 48.6 and 34.6%, respectively, for the two salts, the composition of the residual undecomposed salt in each experiment is identical within experimental error to that of the corresponding non-irradiated D, L-Leu salt standard.

(Insert Table II)

The gas chromatographically determined percent degradation observed for solid D-, L- and D, L-Leu samples are summarized in Table III, where we see that the D, L-Leu has been degraded to an extent intermediate between that of the similarly irradiated D- and L-Leu samples, and to an extent which is roughly calculable from the separate percent decompositions of the individual enantiomers. As in the earlier samples subjected to shorter irradiation (Table III, first two lines), the L-Leu appears to be degraded slightly more extensively than the D-Leu, and the G(-M) values are close to those observed for the corresponding earlier samples.

(Insert Table III)

It has been pointed out (Bonner, 1973a) that for valid application of the enantiomeric marker technique to the determination of percent degradation of enantiomers, the agent causing degradation must not itself induce independent racemization. This condition appeared to prevail in our earlier  $\gamma$ -ray radiolyses of solid Leu, where L- and D, L-Leu were approximately equally decomposed (81.2 and 82.9%, respectively) after identical doses of about  $7 \times 10^8$  rads (Bonner, 1973a). However, it has been reported (Nuclear Chicago Bulletin, 1965) that when tritiated L-Leu, prepared at a radioactivity level of 7 Ci per mmole, was allowed to stand in neutral aqueous solution at 0° for 10 months, it was not only 30% decomposed but also 36% racemized. It thus appeared necessary to determine if our present D- and L-Leu had racemized to any significant extent while undergoing the degradations indicated in Table III. This was accomplished by conversion of the irradiated samples Nos. 2 and 3 (Table I) into their N-TFA-(+)-2-butyl esters, then comparing their GC-determined enantiomeric compositions with those of corresponding non-irradiated

Table II  
<sup>90</sup>Sr RADIOLYSIS OF D, L-LEUCINE Na AND  
HCL SALTS IN AQUEOUS SOLUTION<sup>a</sup>

Sample	Number <sup>b</sup>	% Decomposed	Enantiomeric Composition			
			% D	% L	(±) <sup>c</sup>	No. of Analyses <sup>d</sup>
Na Salt	10	48.6 <sup>d, f</sup>	49.41	50.59	0.30	3
Na Salt standard <sup>e</sup>	-	-	49.26	50.74	0.19	3
HCl salt	11	34.6 <sup>d, g</sup>	50.33	49.67	0.53	3 <sup>h</sup>
HCl salt standard <sup>e</sup>	-	-	50.28	49.72	0.10	4

a. 61.7 KCi <sup>90</sup>Sr-<sup>90</sup>Y; 1.34 years; 4.11 x 10<sup>8</sup> rads. b. In Table I. c.

All (±) values reported in this paper represent standard deviations. d.

Analytical method: GC-1. e. Non-irradiated. f. Based on 7 GC analyses having ± 0.31%. g. Based on 3 GC analyses having ± 0.27%.

h. A small impurity peak on the tail of the D-Leu-(+)-2-butyl ester peak was included in the electronic integration. The peak areas were accordingly hand integrated (peak height times 1/2-peak width).

Table III  
DEGRADATION ON  $^{90}\text{Sr}$  RADIOLYSIS OF  
SOLID LEUCINE SAMPLES

Leu Isomer	No. <sup>a</sup>	Radiation Exposure		$\%$ Decomposed	(±)%	G(-M)	(±)
		Years	Rads x 10 <sup>-8</sup>				
D	2	0.34	1.05	3.5 <sup>b</sup>	0.3	2.5	0.2
L	3	0.34	1.05	4.9 <sup>b</sup>	1.1	3.4	0.8
D	2	1.34	4.11	12.7 <sup>c</sup>	1.2	2.3	0.2
L	3	1.34	4.11	16.2 <sup>c</sup>	1.0	3.0	0.3
D, L	1	1.34	4.11	13.7 <sup>d</sup>	1.4	2.5	0.3
D, L- (Calcd) <sup>e</sup>	-	1.34	4.11	(14.4) <sup>e</sup>	-	(2.6) <sup>e</sup>	-

a. In Table I. b. Based on 3 GC analyses using the N-TFA-L-prolyl diastereomer method (Bonner, 1972b). c. Based on 4 GC analyses using Method GC-1 and 4 using Method GC-4. d. Based on 3 GC analyses using Method GC-1. e. Calculated from the individual % decompositions of the D-(12.7%) and L-(16.2%) enantiomers above.

samples. The analytical results, summarized in Table IV, indicate that only very trivial racemization attended the irradiation of either crystalline Leu enantiomer during the 1.34 year period involved. Thus the percent decomposition data for Nos. 2 and 3 in Table II should be reasonably accurate.

(Insert Table IV)

We turn finally to the critical experiment in this series, namely, determination of the enantiomeric composition of the irradiated D, L-Leu. Assuming the validity of the 12.7% and 16.2% degradations, respectively, for the individual D- and L-Leu enantiomers (Table III), one can calculate that the irradiated D, L-Leu should have an enantiomeric composition of 51.0% D- and 49.0% L-Leu. This difference is close to, but hopefully a bit beyond, the experimental error of our GC analytical technique. We accordingly undertook these GC analyses with particular care, using method GC-1 at the outset. The results of these and subsequent analyses, along with the corresponding non-irradiated D, L-Leu controls, are summarized in Table V.

(Insert Table V)

In the two April, 1973 sets of analyses it appeared as if the L-Leu was in fact undergoing radiolysis slightly more rapidly than the D-Leu, in that the differences in enantiomeric compositions of the irradiated sample and the control were slightly beyond the standard deviation in each set of analyses. These results were reported tentatively in mid-1973 (Bonner, 1973b). To confirm these results the samples were subsequently subjected to additional GC analyses, using different columns, derivatives and GC parameters. In these analyses of August, October and November, 1973, however, the enantiomeric compositions of the irradiated and non-irradiated samples proved identical within experimental error. This same conclusion was evident on averaging all of the GC analyses conducted on these samples for enantiomeric composition (Table V). We thus find no evidence using GC criteria for the induction of optical activity in our irradiated solid D, L-Leu.



Table IV  
TEST FOR RACEMIZATION IN  
IRRADIATED D- AND L-LEUCINE

Leu Isomer	Irradiated <sup>a</sup>				Non-irradiated				Difference	
	% D	% L	(±)	No. of Anal. <sup>b</sup>	% D	% L	(±)	No. of Anal. <sup>c</sup>	% <sup>d</sup>	
D	98.81	1.19	0.23	3	99.50	0.50	0.24	6	0.69	
L	1.05	98.95	0.28	3	0.60	99.40	0.19	7	0.45	

a. 61.7 KCi <sup>90</sup>Sr-<sup>90</sup>Y; 1.34 years;  $4.11 \times 10^8$  rads. b. Analytical method:

GC-2. c. Analytical method: GC-1. d. % of main isomer (non-irradiated) -

% of main isomer (irradiated).

Table V  
REPLICATE GC ANALYSES FOR ENANTIOMERIC  
COMPOSITION OF IRRADIATED D,L-LEUCINE AND CONTROL

<u>Date</u>	<u>%D</u>	<u>%L</u>	<u>(±)</u>	<u>No. of Anal.</u>	<u>%D</u>	<u>%L</u>	<u>(±)</u>	<u>No. of Anal.</u>	<u>Difference<sup>b</sup> %</u>
4/26/73	50.81	49.19	0.07	3 <sup>c</sup>	50.24	49.76	0.19	3 <sup>c</sup>	0.57
4/27/73	50.53	49.47	0.24	4 <sup>c</sup>	49.87	50.13	0.34	4 <sup>c</sup>	0.66
8/9/73	50.18	49.82	0.27	5 <sup>d</sup>	50.31	49.69	0.39	5 <sup>d</sup>	-0.13
10/24/73	50.64	49.36	0.16	3 <sup>e</sup>	50.41	49.59	0.19	3 <sup>e</sup>	0.23
11/13/73	50.13	49.87	0.48	4 <sup>f</sup>	50.15	49.85	0.32	6 <sup>f</sup>	-0.02
Average <sup>g</sup>	50.42	49.58	0.29	19	50.18	49.82	0.31	21	0.24
Theo- retical	51.0 <sup>h</sup>	49.0 <sup>h</sup>	-	-	50.0	50.0	-	-	1.0

a. 61.7 KCl  $^{90}\text{Sr}$ - $^{90}\text{Y}$ ; 1.34 years;  $4.11 \times 10^8$  rads. b. %D (irradiated)-  
 %D (control). c. Method: GC-1. d. Method: GC-2. e. Method: GC-3.  
f. Method: GC-4. g. Weighted for number of analyses. h. Calculated from  
 individual % decomposition of D- and L-Leu (Table III).

## DISCUSSION

In the experiments described above, using both ORD and GC criteria, we have thus found no evidence for the asymmetric  $\beta$ -ray Bremsstrahlung radiolysis of racemic amino acid samples (either solid or in solution), even with the powerful 61.7 KCi  $^{90}\text{Sr}$ - $^{90}\text{Y}$  source and the extensive 1.34 year irradiation period employed. The failure to note asymmetric decomposition of D, L-Leu salts in aqueous solution (Table II) was perhaps to be expected since, as mentioned above, symmetrical solvent photochemistry or radiochemistry would presumably be intimately involved in the mechanism leading to the extensive degradations observed.

Since no asymmetric degradation was observed in the case of solid D, L-Leu either, one might question the 3.5% difference noted in Table III for the percent decomposition of the individual D- and L-Leu enantiomers. There are several conceivable causes (beyond gross experimental error) which might engender this difference. The first of these involves radiation field inhomogeneities in the  $^{90}\text{Sr}$  source itself. Fig. 1 indicates that the four concentrically located  $^{90}\text{Sr}$  cans constituting the source were not of exactly identical radioactivity content, but varied from 14.1 to 18.8 KCi. Furthermore, our sample container was merely placed in the approximate center of the array, and no provision could be made to insure an absolutely uniform dose rate to each of the 21 individual sample vials irradiated. The 28% greater decomposition of L-Leu over D-Leu, however, seems rather too large to be accommodated by this explanation alone. Secondly, trace impurities of different sorts in the separate D- and L-Leu isomers could conceivably lead to overall differences in radiation susceptibility with consequent variations in the G(-M) values, since the samples irradiated were top-grade commercially available ones used without additional purification. Third, since no attempt was made to insure sample vial uniformity and since  $^{90}\text{Sr}$  has a  $\beta$ -particle energy of only 0.54 Mev (only 3.46 times the energy of  $^{14}\text{C}$   $\beta$ -particles, which are completely stopped by glass) (Heath, 1968), it is clear that random variations in the wall thicknesses of our glass sample vials could affect the total radiation doses received by the vial contents, and hence their

extents of radiolysis. Finally, it is conceivable that the G(-M) value for radiolysis of D-Leu (or L-Leu) might be different in a D-Leu (or L-Leu) crystal lattice than in a D, L-Leu crystal lattice. That is, asymmetric degradation might be observable in a simple D, L mixture (conglomerate), but might not occur in a racemic compound or a racemic solid solution. The latter situation might occur if radiolysis of the D- or L-isomer in the same crystal lattice could give rise to an intermediate which then somehow engendered extensive further degradation (chain type reaction) of the D- and L-molecules in that same crystal lattice with equal probability. This perhaps remote possibility is under current experimental investigation.

In addition to the latter remote possibility, there is in retrospect an even more fundamental reason why perhaps the sought after asymmetric radiolysis of racemic solid samples has not been observed and might indeed not be observable.  $\beta$ -ray Bremsstrahlung are emitted with a spectrum of energies ranging from quite low up to values approaching the original energy of the  $\beta$ -decay electron. However, the vast majority of the Bremsstrahlung intensity lies in the low energy region of this spectrum (Wyrd, 1955). On the other hand, it is only the sparse very high energy end of the spectrum which produces completely circularly polarized Bremsstrahlung photons, while the circular polarization of the photons produced at the predominant low energy end of the spectrum drops off rapidly as we go to lower energies (Schopper and Galster, 1958; Goldhaber et al., 1957; McVoy, 1957). Thus the major portion of the Bremsstrahlung radiation consists of both low energy as well as poorly circularly polarized photons. Furthermore, it seems most probable that these predominant low energy, poorly polarized photons would be the very ones which should bring about the hopefully asymmetric radiolyses we are trying to observe, since these energies are closer, at least, to the radiation energies which ordinarily bring about photochemical changes in molecules. Thus it seems likely that the radiolyses which we did observe were brought about by the most abundant and least circularly polarized photons in the Bremsstrahlung spectrum, and that asymmetric effects depending on the circular polarization of these Bremsstrahlung would therefore be at a minimum, and perhaps intrinsically unobservable.

There are two ways in which this dilemma might hopefully be circumvented. One is to use longer irradiation times, such that the extent of degradation will be greater and any small asymmetric bias might become magnified. To this end, we plan to leave the third can of samples in the  $^{90}\text{Sr}$  source at Oak Ridge for several more years, if practical. The second approach is to use an alternative and more powerful source of longitudinally polarized  $\beta$ -rays, namely, those from an appropriately modified linear electron accelerator. Preliminary pilot experiments on amino acid degradation with the unmodified accelerator have already been described (Bonner, 1973a), and experiments with the longitudinally polarized electron beam from the modified accelerator are currently in progress. We hope that these two lines of investigation may eventually contribute a definitive answer to the validity of the parity violation  $\beta$ -decay mechanism for the origin of optical activity in nature.

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## REFERENCES

- Akabori, S., Okawa, K., Sato, M.: Introduction of side-chains into polyglycine dispersed on solid surface. I. Bull. Chem. Soc. Japan, 29, 608 (1956).
- Bernstein, W. J., Calvin, M., Buchardt, O.: Hindered rotation about arylethylene-bonds in the excited state of diarylethylenes. Structural effects on the asymmetric synthesis of 2- and 4-substituted hexahelicenes. J. Am. Chem. Soc., 95, 527 (1973).
- Bonner, W. A.: Origins of Molecular Chirality. In: Exobiology (ed. C. Ponnamperna), pp. 117-181. Amsterdam: North-Holland Publishing Co. (1972a).
- Bonner, W. A.: The adaptation of diastereomeric S-prolyl dipeptide derivatives to the quantitative estimation of R- and S-leucine enantiomers. J. Chromat. Sci., 10, 159 (1972b).
- Bonner, W. A.: Enantiomeric markers in the quantitative gas chromatographic analysis of optical isomers. Application to the estimation of amino acid degradation. J. Chromat. Sci., 11, 101 (1973a).
- Bonner, W. A.: Experiments on the origins of optical activity, Address to the "4th International Congress on the Origin of Life," Barcelona, Spain, June 1973b.
- Elad, D., Sinnreich, J.: Photoalkylation of Glycine Derivatives. Chem. Communications, 472 (1965).
- Feibush, B.: Interaction between asymmetric solutes and solvents. N-Lauroyl-L-valyl-t-butylamide as a stationary phase in gas liquid partition chromatography, Chem. Communications, 544 (1971).
- Fox, S. W.: The development of rigorous tests for extraterrestrial life. In: Biology and the Exploration of Mars (eds. Pittendrigh, C. S., Vishniac, W., Pearman, J. P. T.), pp. 213-228. Washington, D. C.: Nat. Acad. Sci.-Nat. Res. Council, 1966.
- Garay, A. S.: Origin and role of optical isomerism in life. Nature 219, 338 (1968).
- Gil-Av, E.: Present status of enantiomeric analysis by GC. Addresses to the "4th International Congress on the Origin of Life," Barcelona, Spain, June, 1973, and to the "International Symposium on Generation and Amplification of Asymmetry in Chemical Systems," Julich, Fed. Rep. Germany, September 1973.

- Gul'danskii, V. I., Khrapov, V. V.: Comparison of the effect of electron irradiation on the optical activity of racemates and optical antipodes. Soviet Physics JETP 16, 582 (1963).
- Goldhaber, M., Grodzins, L., Sunyar, A. W.: Evidence for circular polarization of Bremsstrahlung produced by beta rays. Phys. Rev. 106, 826 (1957).
- Halpern, B., Westley, J. W., Levinthal, E. C., Lederberg, J.: The Pasteur probe: An assay for molecular asymmetry. Life Sci. and Space Res., 5, 239 (1966).
- Heath, R. L.: Table of the Isotopes. In: R. C. Weast, Ed. Handbook of Chemistry and Physics. 49th Edition, pp. B-7, B-26. Cleveland, Ohio: The Chemical Rubber Co. 1968.
- Kagan, H., Moradpour, A., Nicoud, J. F., Balavoine, G., Martin, R. H., Cosyn, J. P.: Photochemistry with circularly polarized light. II. Asymmetric synthesis of octa- and nonahelicene. Tetrahedron Lett., 2479 (1971).
- Kuhn, W., Braun, E.: The photochemical origin of optically active substances, Naturwiss., 17, 227 (1929).
- Kuhn, W., Knopf, E.: Preparation of optically active substances with the help of light. Z. physik. Chem., 7B, 292 (1930a).
- Kuhn, W., Knopf, E.: Photochemical preparation of optically active substances, Naturwiss., 18, 183 (1930b).
- Lee, T. D., Yang, C. N.: The question of parity conservation in weak interactions. Phys. Rev. 104, 254 (1956).
- McVoy, K. W.: Circular polarization of Bremsstrahlung from polarized electrons in Born approximation. Phys. Rev., 106, 828 (1957).
- Meister, A.: Biochemistry of the Amino Acids, Vol. I, 2nd edition, pp. 141-144. New York: Academic Press 1965.
- Nuclear-Chicago. The stability of labelled organic compounds. Radiochemical Division, Nuclear-Chicago, Des Plaines, Illinois 60018, August, 1965.
- Paschke, R., Chang, R. W. H., Young, D.: Probable role of gamma irradiation in origin of life. Science, 125, 881 (1957).
- Pollock, G. E., Oyama, V. I., Johnson, R. D.: Resolution of racemic amino acids by gas chromatography, J. Gas Chromatog., 3, 174 (1965).

- Schopper, H., Galster, S.: The circular polarization of internal and external Bremsstrahlung. Nuclear Phys., 6, 125 (1958).
- Stryer, L.: Optical Asymmetry. In: Biology and the Exploration of Mars (eds. Pittendrigh, C. S., Vishniac, W., Pearman, J. P. T.), pp. 141-146, Washington, D. C.: Nat. Acad. Sci.-Nat. Res. Council, 1966.
- Tolbert, B. M., Krinks, M. H., Castrillon, J. A., Henderson, L. E., Finch, M. B.: Radiation chemistry of crystalline amino acids. (Unpublished manuscript); 1962.
- Ulbricht, T. L. V.: Asymmetry: The non-conservation of parity and optical activity. Quart. Rev., 13, 48 (1959).
- Ulbricht, T. L. V.: The origin of optical activity and the origin of life. In: Comparative Biochemistry, Vol. IV, Part B (eds. Florkin, M., Mason, H. S.), pp. 16-25, New York: Academic Press, 1962.
- Ulbricht, T. L. V., Vester, F.: Attempts to induce optical activity with polarized  $\beta$ -radiation. Tetrahedron, 18, 629 (1962).
- Vester, F.: Seminar at Yale University, Feb. 7, 1957.
- Vester, F., Ulbricht, T. L. V. Krauch, H.: Optical activity and parity violation in  $\beta$ -decay. Naturwiss., 46, 68 (1959).
- Wu, C. S., Ambler, E., Hayward, R. W., Hoppes, D. D., Hudson, R. P.: Experimental test of parity conservation in  $\beta$ -decay. Phys. Rev., 105, 1413 (1957).
- Wyard, S. J.: Radioactive-source corrections for Bremsstrahlung and scatter. Nucleonics, 13, 44 (1955).